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Ca^{2+}- and Mg^{2+}-ATPases in the Golgi Apparatus and Microsomes of the Lactating Mammary Glands of Cows

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During lactation, the secretory cells of the mammary gland transport large amounts of calcium from blood into milk. In a high-producing dairy cow the total calcium content of blood is 2.5–3 mM, whereas that of milk is approximately 31 mM.[1] Thus, a tenfold increase in the concentration of calcium is achieved. Two thirds of the calcium is bound to casein, which results in the formation of a colloidal complex, the casein micelle; most of the rest (~ 7 mM) is bound to citrate, and small amounts (~ 2 mM) are present as free ions.[1] It is thought that a calcium pump is involved in the accumulation of Ca^{2+} in the Golgi apparatus. ATPase activity has been studied in the Golgi-enriched fractions from bovine mammary gland.[2] The ATPase activity required Mg^{2+} and was stimulated by Ca^{2+} and KCl. In addition to ATPase activity, mammary Golgi membranes have the ability to accumulate calcium. Evidence for this calcium pump activity was obtained from studies on cows,[2] mice,[3] and rats.[4,5]

This study deals with the divalent cation-stimulated ATPase activity in lactating mammary glands of cows. The ATPase activity in microsomal and Golgi apparatus membranes is investigated to clarify their roles in Ca^{2+} transport through the endo membrane system of the lactating mammary gland and to better understand the mechanism involved in the transport of Ca^{2+} into milk.

Mammary glands were obtained from cows in full lactation through the cooperation of the Beltsville Agricultural Center. The glands were trimmed of extraneous fat, sliced, and stored at −80°C. Microsomes and Golgi apparatus were prepared from frozen mammary gland and identified by marker enzymes as previously described.[6]

The ATPases in the microsomes and the Golgi apparatus required Ca^{2+} or Mg^{2+} for activity (FIG. 1); very little activity occurred in the absence of these cations. In the presence of Ca^{2+}, the ATPase activities increased with the concentration of Ca^{2+} (FIG. 1A). When the effect of Mg^{2+} was tested, both membrane ATPase activities obtained maximum activity between 0.02 and 0.03 mM Mg^{2+}; the activity declined at higher Mg^{2+} concentrations (FIG. 1B).

ATPases involved in ion translocation have been classified into three groups (P, F, and V) by Pedersen and Carafoli.[8] The mammary ATPases (Golgi apparatus and microsomes) can be characterized by examining the effect of various reagents on activity (TABLE 1). The mammary ATPases would be classified as the P type, because vanadate was a more potent inhibitor than *N*-ethylmaleimide and oligomycin, which

are inhibitors of V- and F-type ATPase.[8] The lack of inhibition by ouabain and *N*-ethylmaleimide suggests that the enzymes differ from the plasma membrane [Na^+ + K^+]-ATPases. Quercetin, an inhibitor of sarcoplasmic reticulum and red cell plasma membrane ATPase, did not appreciably inhibit the mammary enzymes.[3] The mammary ATPases, like most Ca^{2+}-ATPases, were inhibited by La^{3+}. The detergents, Triton X-100 and *n*-octyl β-D-glucopyranoside, inhibited the microsomal ATPase and had less effect on the Golgi ATPase activity. Whether these differences

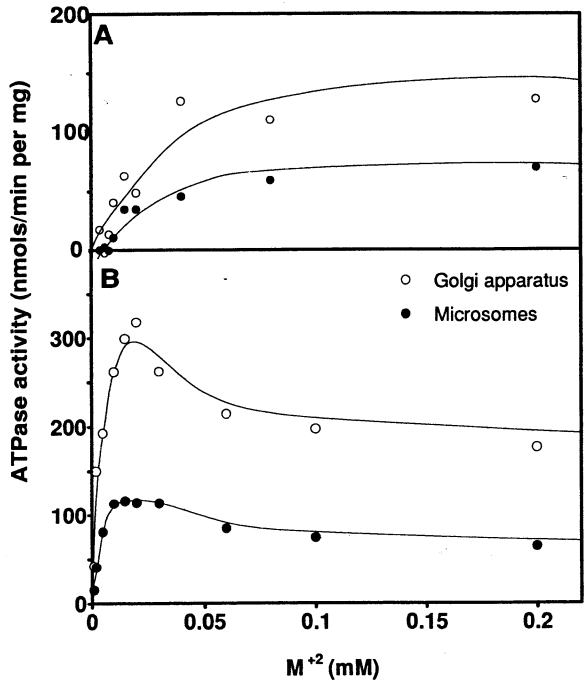

FIGURE 1. Dependence of ATPases (Golgi apparatus and microsomes) on free Ca^{2+} (**A**) and free Mg^{2+} (**B**). The effect of Ca^{2+} on ATPase activity was determined in the presence of 0.4 mM Ca^{2+}-ATP and 0.4 mM Ca^{2+}-EGTA. Ca^{2+} (free) was varied from 4–200 μM. The effect of Mg^{2+} on ATPase activity was determined in a similar manner. Activity was measured in the presence of 0.4 mM Mg^{2+}-ATP, 0.01 mM mg-EGTA, and free Mg^{2+} (1–200 μM). ATPase activities were determined on 0.5 ml of reaction mixture as described in TABLE 1 with membrane preparations that were dialyzed for 48 hours against 30 m*M* MOPS buffer pH 7.4.

can be attributed to differences in the enzyme molecules or to the membrane environment cannot be determined at the present time.

The translocation of calcium from blood to milk could be supported by the ATPase activities described in this report. The microsomal activity, which contains endoplasmic reticulum and plasma membrane, with a specific activity of 90 nmol phosphorus per minute per milligram could support Ca^{2+} transfer from the base-

TABLE 1. Effect of Various Reagents on Ca^{2+}- and Mg^{2+}-ATPase Activities in the Lactating Bovine Mammary Gland[a]

Effector	% Activity	
	Golgi Apparatus	Microsomes
None (control)	100	100
Oligomycin (20 μg/ml)	76 ± 9	83 ± 7
Sodium vanadate (0.5 mM)	38 ± 7	30 ± 1
Quercetin (1 mM)	82 ± 3	94 ± 0
N-Ethylmaleimide (0.2 mM)	99 ± 5	94 ± 8
Ouabain (5 mM)	106 ± 9	111 ± 3
$LaCl_3$ (0.1 mM)	11 ± 3	58 ± 4
Triton X-100 (0.5%)	97 ± 1	65 ± 1
n-Octyl glucoside (0.5%)	71 ± 1	27 ± 1

[a]These reagents were included in the assays at the indicated concentrations. Ca^{2+}- and Mg^{2+}-ATPase activities were determined at pH 7.4 in a 0.5-ml reaction mixture containing 0.3 mM Ca^{2+} (free), 0.15 mM Mg^{2+} (free), 0.4 mM Ca^{2+}-EGTA, 0.4 mM Mg^{2+}-ATP, and 50 mM 3-(*N*-morpholino) propane sulfonic acid (MOPS). The reaction was initiated by the addition of 30–50 μg/ml ATPase preparation and incubated for 15 minutes at 37°C. The inorganic phosphate was determined by the addition of a malachite green-molybdate-polyvinyl alcohol reagent, as described by Chan *et al.*[7]

ment membrane to cytosol. The Golgi enzyme has a specific activity of 125 nmol phosphorus/min per mg and could function to transfer Ca^{2+} into secretory vesicles.

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